

Increased Vascular-Endothelial Growth Factor (VEGF) Tumor Expression and Response to Epidermal Growth Factor Receptor (EGF-R) Inhibitor Erlotinib in Non-small Cell Lung Cancer (NSCLC)

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A 37-year-old female never smoker with metastatic large cell carcinoma of the lung had a partial response to a second line palliative therapy with the EGF-R tyrosine kinase inhibitor erlotinib after platinum based first line therapy failed. Molecular analysis of the primary and a liver metastasis did neither find any EGF-R mutation nor an EGF-R amplification. However, both the primary and the metastasis showed an increased gene expression of vascular-endothelial growth factor-A in contrast to normal tissue, which was confirmed by immunohistochemistry. To our knowledge, this is the first report about a high vascular-endothelial growth factor-A expression in the tumor of a patient responding to an EGF-R inhibitor postulating that there might be a link between both tyrosine kinase pathways.

Key Words: Non-small cell lung cancer (NSCLC), Epithelial growth factor receptor (EGFR), Vascular-endothelial growth factor (VEGF), Response to chemotherapy.

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A 37-year-old Caucasian woman presenting with a 6-month history of coughing with hemoptysis and a weight loss of 5 kg in 6 months was admitted to our hospital on October 3, 2005. The patient has never smoked. In the flexible bronchoscopy the left segmental ostium 9 was closed. Snap biopsies of this region revealed a low-grade large cell NSCLC. Furthermore, a centigram of the bones suspected bone metastases at the left iliac spine, confirmed by an MRT

of the pelvis. The investigations were completed by CT scans of the thorax and abdomen, which resulted in an UICC IV tumor stage.

The patient was given palliative first line chemotherapy, consisting of carboplatin 300 mg/m² and docetaxel 75 mg/m² on days 1 and 22. On staging CT scans progressive disease was evident with an increased primary tumor (Figure 1A; arrows) with increased and additional metastases in the liver (Figure 1C). Additionally, brain metastases were present on cranial CT. Cranial irradiation with a total dose of 50 Gy was applied, beginning on December 8. As the performance status was still acceptable (EGOG-PS 2), a palliative second line oral therapy with the EGF-R tyrosine kinase inhibitor erlotinib (150 mg/d) was started on December 14, 2005. On February 10, 2006, a further tumor staging showed a partial remission of both the primary tumor and at all metastatic sites. Brain metastasis was no longer evident. On April 11, 2006 (4 months erlotinib), staging with CT scans confirmed the partial response (Figure 1B, D). About 10 months after the initial diagnosis of metastatic NSCLC, the patient died with progressive cachexia and dyspnoea due to recurrent pulmonary bleeding on July 1, 2006. Formalin fixed paraffin embedded tissue from biopsies of primary tumor and the liver metastasis taken during initial diagnosis, was laser microdissected. DNA and RNA were isolated separately from the tumor cells and normal epithelial tissue, respectively. Exons 18 to 21 of the EGF-R gene were bidirectionally sequenced and no somatic mutation could be detected neither in the primary nor in the liver metastasis (data not shown).¹ Chromogenic in situ hybridization analysis showed only a low polysomy of the EGF-R according to the categories defined by Cappuzzo et al.² (Figure 2A; original magnification $\times 500$). Additionally, using tumor RNA, gene expression analysis on the EGF-R gene and further members of the epidermal growth factor receptor family genes (Her2/neu, ERBB3, ERBB4) together with four housekeeping genes was performed by RT-PCR. Additionally, as there were hints from metastatic colorectal carcinoma, that there might be interactions between the EGF-R and the vascular-endothelial growth factor (VEGF) ligands,³ the VEGF family gene expressions (VEGF-A, VEGF-B, VEGF-C, and VEGF-D) were exploited by this

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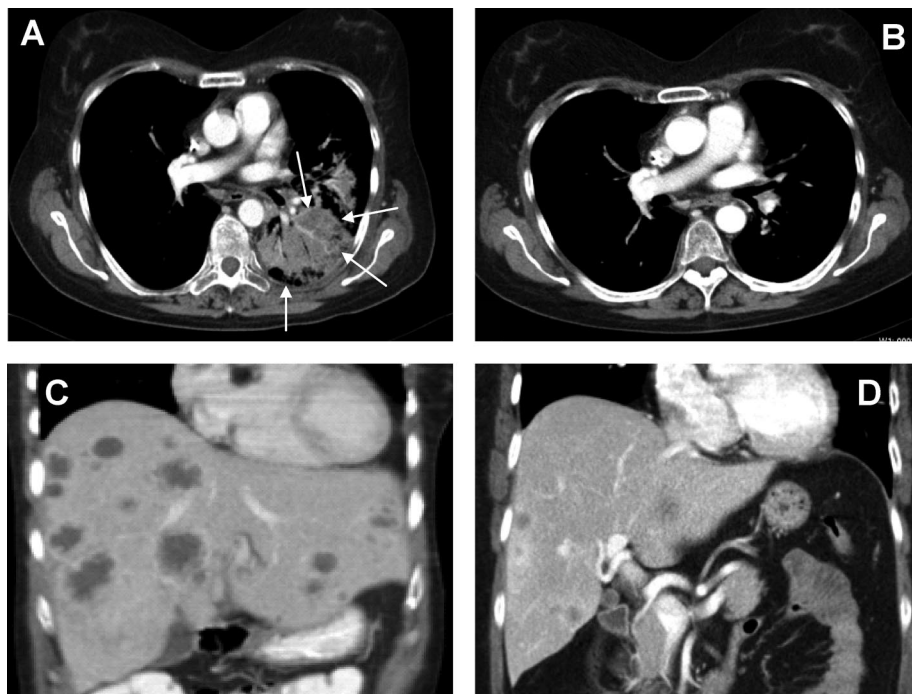


FIGURE 1. *Radiological images before and after erlotinib treatment* Axial, contrast-enhanced, CT images of the lungs show (A) the primary tumor (arrays) in the left lower lobe (December 2005), (B) which shrunk after treatment (April 2006). Additionally, coronal contrast-enhanced CT images of the abdomen, (C) presenting with multiple liver metastases (December 2005), (D) which significantly decreased in size and number after therapy (April 2006).

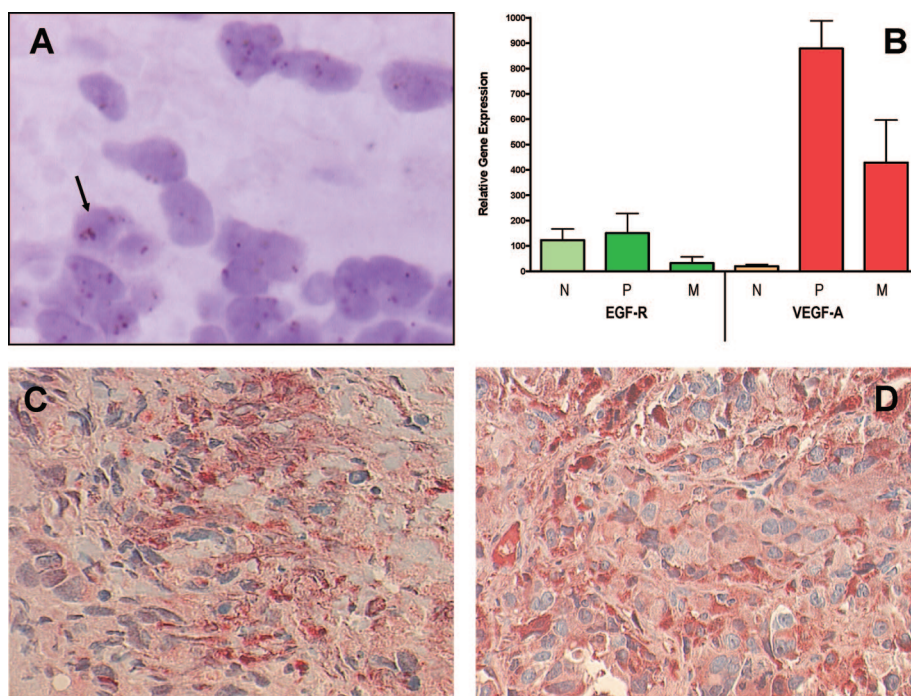


FIGURE 2. *Expression analysis of EGF-R and VEGF in the tumor and liver metastasis* (A) Chromogenic in situ hybridisation (CISH) analysis showing low polysomy (array) of the EGF-R locus in the nuclei of the tumor cells. (B) Results from the expression analysis of the EGF-R and VEGF genes by quantitative real-time polymerase chain-reaction (qRT-PCR) after laser microdissection in normal lung epithelium (N), primary tumor (P) and liver metastasis (M) presenting a high VEGF overexpression in both the tumor and the metastasis. The VEGF overexpression could be confirmed with immunohistochemistry, (C) showing a strong cytoplasmatic staining (dark red) of both the primary lung tumor cells and (D) the liver metastasis using anti-VEGF antibodies.

method, too. Although there was an evident over expression of EGF-R gene in the primary tumor (lower were detected in the liver metastasis), VEGF-A gene was greatly up-regulated in the primary (P) and in the liver metastases (M) in contrast to normal lung epithelial cells (N) (Figure 2B). The level of expression of the other genes did not differ significantly between normal and tumor tissue(s). The VEGF-A over expression was confirmed on the protein level by immunohistochemistry. With

antibodies against VEGF-A (Santa Cruz, CA, at 1:120 dilution) there was a strong cytoplasmatic staining as well in the primary lung tumor (Figure 2C) as in the liver metastasis (Figure 2C, D; original magnification $\times 50$).

DISCUSSION

Angiogenesis has been demonstrated to be an important event in the process of tumor growth and metastatic dissem-

ination. In this context, VEGF is a well-established key regulator of new blood vessel formation. To our knowledge, there is no report published so far about a clinical response of a VEGF over expressing NSCLC to an EGF-R therapy. The question arises, whether VEGF is of predictive relevance in this context. Data from head and neck squamous cell carcinoma cell lines, where EGF-R inhibitors lead to down-regulation of VEGF-A expression casually support our observations.⁴ Furthermore, in metastatic CRC a sudden and long lasting reduction of VEGF serum levels was seen during palliative therapy with cetuximab, an anti-EGF-R antibody.³ To date, the mechanism(s) leading to VEGF down-regulation have not been fully explained. It is hypothesized, that VEGF expression might be either down-regulated by hypoxia inducible factor 1 α , a transcriptional regulator of VEGF expression and/or via the Sp1 binding site of the VEGF promoter.^{4,5} Our hypothesis has some limitations. Firstly, we do not know whether VEGF over expression was still evident at the beginning of the second-line EGF-R therapy, while the biopsy was taken at the time of diagnosis. Secondly, we do not know, whether there was a down-regulation of VEGF in the tumor, as we did not have a biopsy at the time of best response. Thirdly, there is currently some debate concerning the sensitivity and specificity of molecular techniques to identify somatic mutations within the tyrosine kinase domain of the EGF-R gene. As every mutation screening method, direct bidirectional sequencing as used herein, has its limitations. However, this method is well established and to date there is no clear comparison available to indicate the overall

failure rate of any given technology.^{1,6,7} We conclude, that EGF-R therapy might have influence on clinical response by down-regulation of VEGF-A. However, the specific role of VEGF in NSCLC and its importance for treatment decisions remains to be investigated. A retrospective analysis of VEGF-A in tumors of patients responding to erlotinib has been started by our group.

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